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Numerical Analysis of Electrophoretic Protein Patterns of Methicillin-Resistant Strains of *Staphylococcus aureus*

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A total of 50 strains of *Staphylococcus aureus*, including 41 methicillin-resistant *S. aureus* (MRSA) strains, were characterized by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell proteins. The protein patterns contained 40 to 50 discrete bands and were highly reproducible. Partial patterns were used as the basis of a computer-assisted numerical analysis. The MRSA strains clustered into four phenons at the 83% similarity level; and further division of phenon 1, at the 86% similarity level, resulted in a total of six clusters. All of the MRSA isolates from an MRSA epidemic in the United Kingdom were found to cluster in phenon 1 together with 9 of the 12 MRSA isolates from eastern Australia and 3 other MRSA isolates from the United Kingdom. The remaining three eastern Australian isolates clustered separately in phenon 2. Phenon 3 appeared to be exclusive to strains that were both susceptible and resistant to methicillin and that reacted with group V phages, and phenon 4 comprised 11 isolates, all of which were other MRSA isolates from the United Kingdom. We conclude that computer-assisted numerical analysis by high-resolution sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell proteins provides additional criteria for the study of the epidemiology and the evolution of MRSA.

In recent years strains of *Staphylococcus aureus* resistant to methicillin (MRSA) and many other antibiotics have caused increasing problems of nosocomial infections in many parts of the world (25), and certain epidemic MRSA (EMRSA) strains may be more capable of colonizing or infecting patients. One such EMRSA, often termed "the EMRSA," has caused particular problems in southeast England (5), and its type has proven difficult to determine with the International Basic Set of phages (20, 23). A number of alternative typing systems have been evaluated, including a variety of electrophoretic techniques (11).

High-resolution polyacrylamide gel electrophoresis (PAGE) of proteins has been used increasingly in bacterial systematics both at and below the species level and, more recently, for type determination (7, 13, 16). The technique has been applied with success to the identification of strains belonging to four different species of *Staphylococcus*, including *S. aureus* (4). Krikler et al. (17) have used PAGE to separate cellular proteins of *S. aureus* strains but concluded that all isolates were virtually indistinguishable. Both Gaston et al. (11), using conventionally stained gels, and Stephenson et al. (26), using radiolabeled protein profiles, examined strains of MRSA by the sodium dodecyl sulfate (SDS)-PAGE technique in an attempt to distinguish the EMRSA from a number of other MRSA (OMRSA) isolates. Stephenson et al. (26) were able to distinguish the EMRSA from the OMRSA isolates, but Gaston et al. (11) were unable to separate the EMRSA from some of their other epidemic MRSA strains on the basis of their protein patterns alone. However, both studies were limited to visual interpretation of protein profiles, which may have been subjective. Comparisons of the individual protein profiles were made only on samples that were run on the same gel, this being the limit defined by the lack of reproducibility experienced between gels by the investigators mentioned above (11, 26). Such problems handicap the SDS-PAGE technique as a useful epidemiological tool. More recently, whole-cell protein patterns of MRSA isolates separated by SDS-PAGE have been analyzed numerically by using the Dice coefficient (27), but it was concluded that the method did not usefully distinguish between different isolates.

Lacey and Grinstead (18) hypothesized that nearly all MRSA strains have evolved from a single clone, and others have suggested criteria by which a clone might be described (24). Recent studies (28) based on an analysis of plasmids have shown that isolates of the EMRSA from two London hospitals were indistinguishable from epidemic strains isolated in eastern Australia.

The aim of the present study was to compare the high-resolution SDS-PAGE whole-cell protein patterns of a number of isolates of methicillin-susceptible *S. aureus* (MSSA), OMRSA, and the EMRSA by using a computerized analysis of patterns in order to gain an objective evaluation of the technique as a typing tool and to determine whether protein profiles could be used as additional criteria for the description of a clone.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1 together with their sources, alternative strain numbers, phage type, resistogram, and protein phenon type. A number of the strains used in this study have been analyzed previously by the PAGE technique. Isolates 1 to 10 (OMRSA isolates) and 38 to 41 (EMRSA isolates) were the strains used in the study of Stephenson et al. (26), and isolates 46 to 50 were used by Krikler et al. (17). It should be noted that although isolates 17 and 18 are listed as eastern Australian MRSA isolates, they were isolated on arrival at St. Thomas' Hospital, London, England, after an intercountry patient transfer from Melbourne, Australia. The EMRSA strains included isolates with a cryptic plasmid and a chloramphenicol resistance plasmid and three isolates that were either cured (isolate 36) or that lacked (isolates 38 and 40) the

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TABLE 1. Strains analyzed by SDS-PAGE of whole-cell proteins

Study isolate	Other isolate designation	Phenon	Source[a]	Phage type[b]	Resistogram[c]
	Colindale OMRSA				
1	Col. 6077	4	DHI	NT	P, E, G, M, Pr, Eb
2	Col. 6145	1b	DHI	29/52/80	P, T, E, M
3	Col. 6255	4	DHI	29/42E/47/54/75/77/83A/(84)/(85)	P, T, E, G, N, Ch, M
4	Col. 6582	4	DHI	75/84/(85)	P, T, E, G, N, Ch, M, Pr, Eb
5	Col. 6621	4	DHI	75/77/83A/(85)/83C/932	P, T, E, G, N, F, M
6	Col. 6983	4	DHI	6/47/75/77/85/90/932	P, M, Eb
7	Col. 7031	4	DHI	52A/52/80/95/75/83A/(85)	P, T, E, F, N, M
8	Col. 7046	4	DHI	80/85/90/932/(77, I)	P, T, E, M
9	CRF 612	4	DHI	47/53/54/75/77/84/85	P, M
10	NCTC 10442	4	DHI	47/53/54/75/77/84/85	P, T, M
	UMDS OMRSA				
11	STH 1854	3	STH	96	P, M
12	STH 6444	1a	STH	(85)	P, T, E, C, N, F, M, Eb
13	STH 6563	3	STH	96	P, M
14	STH 696	4	STH	53/77/83A/84/(85)/88A/932	P, T, E, G, M
15	STH 4682	1a	STH	932/(75, 85, I)	P, T, E, C, G, N, M
16	GH 19	4	Guys	29/(85)/932	P, T, E, G, N, M, Pr, Eb
	Eastern Australian MRSA				
17	STH 16900	1a	STH	85/932	P, T, E, C, G, M, Pr, Eb
18	STH 16906	1a	STH	85/932/(84, I)	P, T, E, C, G, N, Ch, M, Pr, Eb
19	SK 52	1a	Monash	88A/932	P, T, E, C, G, Ch, M, Pr, Eb
20	SK 429	1a	Monash	88A/932	P, T, E, C, G, Ch, M, Pr, Eb
21	BP 18	2	RPAH	88A/932	P, T, E, C, G, Ch, M, Pr, Eb
22	BP 24/1	2	RPAH	(85)/88A/932	P, T, E, C, G, M, Pr, Eb
23	BP 14	2	RPAH	83C/932/(84, I)	P, T, E, C, G, M, Pr, Eb
24	BP 35	1a	RPAH	83C/932	P, T, E, C, G, M, Pr, Eb
25	PB 30	1b	RPAH	88A/90/(83C)/932/(85, I)	P, T, E, C, M, Pr, Eb
26	BP 23	1c	RPAH	NT	P, T, E, C, G, N, M, Pr, Eb
27	BP 24/6	1a	RPAH	NT	P, T, E, C, G, N, M, Pr, Eb
28	BP 25	1c	RPAH	NT	P, T, E, C, G, M, Pr, Eb
	The EMRSA				
29	STH 11[CY d]	1a	WCH	(85)/88A/932	P, T, E, C, G, M, Pr, Eb
30	STH 33[CY]	1a	Nuneaton	83A/84/85/88A/932	P, T, E, C, G, M, Pr, Eb
31	STH 20	1a	STH	(85)/88A/932	P, T, E, C, G, Ch, M, Pr, Eb
32	STH 28	1a	STH	(85)/88A/932	P, T, E, C, G, Ch, M, Pr, Eb
33	STH 40254	1a	STH	(85)/88A/932	P, T, E, C, G, M, Pr, Eb
34	STH 4250[CY]	1a	STH	(85)/88A/932	P, T, E, C, G, M, Pr, Eb
35	STH 4696	1a	STH	(85)/88A/932	P, T, E, C, G, M, Pr, Eb
36	BDC 5971	1a	STH	(85)/88A/932	P, T, E, C, M
37	STH 23	1a	STH	(85)/88A/932	P, T, E, C, G, Ch, M, Pr, Eb
38	Col. 5528	1b	DHI	(85)/88A/932/(83C, I)	P, T, E, C, M
39	Col. 6195	1a	DHI	(85)/88A/932/(77, I)	P, T, E, C, G, M, Pr, Eb
40	Col. 5518	1a	DHI	(85)/88A/932/(84, 77, I)	P, T, E, C, G, M, Pr, Eb, R
41	Col. 5671	1a	DHI	(88A)	P, T, E, C, M
	MSSA				
42	STH 1647		STH	NT	P
43	NCTC 6571		STH	52/52A/79/80	
44	NCTC 7121		SS	83C	P
45	4972		SS	52/81/(29, I)	P
46	9/N/37	3	Aberdeen	94/96/D16/47A/27/33	P
47	12/N/33	3	Aberdeen	94/96/D16/47A/27/33	P
48	28/N/20	1b	Aberdeen	29/47/54/75/77/85/81	P
49	28/N/29	1b	Aberdeen	29/47/54/75/77/85/81	P
50	28/N/38	1b	Aberdeen	29/47/54/75/77/85/81	P

[a] Source abbreviations: Aberdeen, T. Pennington, University of Aberdeen, Aberdeen, Scotland; DHI, R. Marples, Division of Hospital Infection, Central Public Health Laboratory, London, England; Guys, S. Poston, Guys Hospital, United Medical and Dental Schools, London, England; Monash, J. Tennent, Monash University, Victoria, Australia; Nuneaton, D. Thomas, Nuneaton Hospitals, Nuneaton, England; RPAH, M. Beard-Pegler and A. Vickery, Royal Prince Alfred Hospital, Sidney, Australia; SS, I. Lind, Statens Seruminstitut, Copenhagen, Denmark; STH, St. Thomas' Hospital, United Medical and Dental Schools, London, England; WCH, K. Cann, Westminster Children's Hospital, London, England.

[b] Abbreviations: NT, Nontypeable, including experimental phages; (*x*, *y*, I), inhibition reactions with, e.g., phages *x* and *y*; parentheses indicate a weak reaction.

[c] Resistogram abbreviations: P, penicillin; T, tetracycline; E, erythromycin; C, clindamycin; G, gentamicin; N, neomycin; Ch, chloramphenicol; F, fusidic acid; M, methicillin; Pr, propamidine isothionate; Eb, ethidium bromide; R, rifampin.

[d] CY, Cryptic plasmid of ca. 1 MDa.

plasmid for gentamicin resistance and resistance to nucleic acid-binding compounds (6).

Phage typing, resistotyping, and lysogenization. Phage typing was performed with the International Basic Set and additional experimental phages at the Staphylococcus Reference Laboratory, Division of Hospital Infection, Central Public Health Laboratory, London, England.

The susceptibilities of the isolates to the agents listed in Table 1 were tested by the standard disk diffusion methods described previously (6, 28).

Four EMRSA strains were lysogenized by the criteria of Gorill and Gray (12), with J phage derived by mitomycin C induction (10) from strain WBG 3358 (28). The phage types of the strains were determined again after successful lysogenization.

Preparation of protein samples. For each culture, a loopful of overnight growth from a blood agar plate was suspended in 20 ml of brain heart infusion broth and incubated at 37°C for 17 h on an orbital shaker. Cells were harvested by centrifugation (11,600 × *g*, 10 min) and washed twice in 10 mM sodium phosphate buffer (pH 7.2). The resulting pellet was suspended in 1 ml of 10 mM sodium phosphate buffer containing 60 μg of lysostaphin and incubated at 37°C for 1 h. The lysate was then heated at 55°C for 10 min to stop the action of lysostaphin. Protein samples were then extracted as described previously (8, 9). The protein content was estimated by the method of Bradford (3) and adjusted to give a final concentration of 30 μg for each sample loading of 17.5 μl.

Electrophoresis. Discontinuous gels (16 by 18 by 0.15 cm) were cast to allow for 10 mm of stacking gel. To give a final polyacrylamide content of 10%, the separation gel was prepared from a stock solution which contained 29.2% (wt/vol) acrylamide and 0.8% (wt/vol) *N,N'*-methylenebisacrylamide (30% T, 2.7% C_{bis}). The final concentrations of other components in the gel were 0.375 M Tris hydrochloride (pH 8.8) and 0.1% (wt/vol) SDS. Polymerization was achieved by adding 0.05% (wt/vol) ammonium persulfate (10% solution) and 0.05% (vol/vol) *N,N,N',N'*-tetramethylethylenediamine. The separation gel components, excluding SDS, were deaerated for 3 min before polymerization, which took approximately 15 min at 20°C.

The stacking gel was prepared from the same stock solution that was used for the separation gel, to give a polyacrylamide content of 5%. The remaining stacking gel components, including the stacking gel buffer, were added to give a final concentration of 0.125 M Tris hydrochloride (pH 6.8) and 0.1% (wt/vol) SDS. Polymerization was initiated by adding 0.05% (wt/vol) ammonium persulfate (10% solution) and 0.1% (vol/vol) *N,N,N',N'*-tetramethylethylenediamine. The stacking gel solution excluding SDS was deaerated for 3 min. Polymerization was evident after 6 to 8 min. The well-forming comb was removed from the stacking gel after 1 h, and the sample wells were washed twice with tank buffer (0.25 M Tris [pH 8.3], 0.192 M glycine, and 0.1% [wt/vol] SDS). The wells were loaded with 17.5 μl of the protein sample to which 0.001% (final concentration; wt/vol) of bromophenol blue was added as a marker dye.

Electrophoresis was carried out for about 4 h in a double-slab vertical electrophoresis cell (Protean; Bio-Rad Laboratories, Richmond, Calif.) at a constant current of 30 mA per gel until the marker dye migrated 100 mm along the length of the separation gel. A constant temperature of 10°C was maintained throughout the electrophoresis with a refrigerated recirculator. After electrophoresis, the gels were stained for 16 h in a solution containing 0.1% (wt/vol) Page Blue 83 in 25% methanol and 10% acetic acid in water (vol/vol/vol). Gels were destained with the same solution but without the stain until the background was clear, and then the gels were dried between dialysis membrane sheets.

Scanning of gels. The stained protein patterns in the dried gels were scanned with a laser densitometer (2222 Ultroscan XL; Pharmacia-LKB Biotechnology, Uppsala, Sweden). The absorbance was recorded at 160-μm intervals along the gel, yielding 625 values per 10-cm gel. The absorbance range was set from 0.15 to 0.8 absorbance units (full scale). A rectangular line beam (800 by 50 μm) was used to scan each lane three times (with no overlap in scan positions), resulting in a multiple lane scan with a width of 2.4 mm. The peak area output for each zone in the sample lane was the mean absorbance of the area scanned. An RS232C and an interface subprogram (RECORD) were used to record the resulting values in absolute ASCII as raw data on a magnetic disk. A recording integrator (2220; Pharmacia-LKB Biotechnology) was used to calculate the retention times and total areas of each protein band.

Analysis and computation of similarity. The initial (stacking gel-separation gel interface) and final (bromophenol blue marker) bands were deleted, and a bacterial standard (strain STH 4696; isolate 35 in Table 1) that was run on each of the gels and was used to correct protein patterns for gel-to-gel variation. A single pattern of the standard strain was designated as the reference pattern. The standard strain was used on the other gels to calibrate patterns against the reference pattern. Segmented linear correction was performed from a total of 19 discernible marker positions (these were usually peaks or valleys) on the reference pattern and by marking the same positions on the calibration pattern replicates. Linear correction (expansion or compression) to the reference distances was carried out within each of the 18 defined segments for each lane on the calibrated gel by three-point quadratic interpolation (14). The length-corrected traces on the reference gel (the gel containing the standard strain reference pattern) were composed of 556 absorbance values after removal of the initial and final bands. A general background trend in each trace was removed to increase discrimination among patterns (14). The background cutoff was set at 0.4. Similarity calculations were based on the partial patterns defined by molecular sizes of 18 to 38.5 kilodaltons (kDa) (which made up approximately 40% of the total protein profile), in which the majority of the qualitative differences were discernible. The similarity between all possible pairs of traces was expressed as the Pearson product moment correlation coefficient, which was converted for convenience to a percent value. The best fit between each pair of traces was obtained by laterally shifting one corrected trace with respect to the other in single-point steps of 160 μm up to 5 points on either side of the initial alignment. Strains were then clustered by the method of unweighted pair group average linkage (UPGMA). Recording of the raw data, segmented linear correction, background trend removal, calculation of similarity, and clustering were all carried out on a microcomputer (Compaq 386) with a program package written in Turbo Pascal (14, 22).

RESULTS

General features of protein patterns by PAGE. One-dimensional SDS-PAGE of whole-cell protein extracts of the 50 *S. aureus* cultures (excluding replicate cultures of eight strains) produced patterns containing 40 to 50 discrete bands with molecular sizes of 18 to 100 kDa. Proteins of <18 kDa were

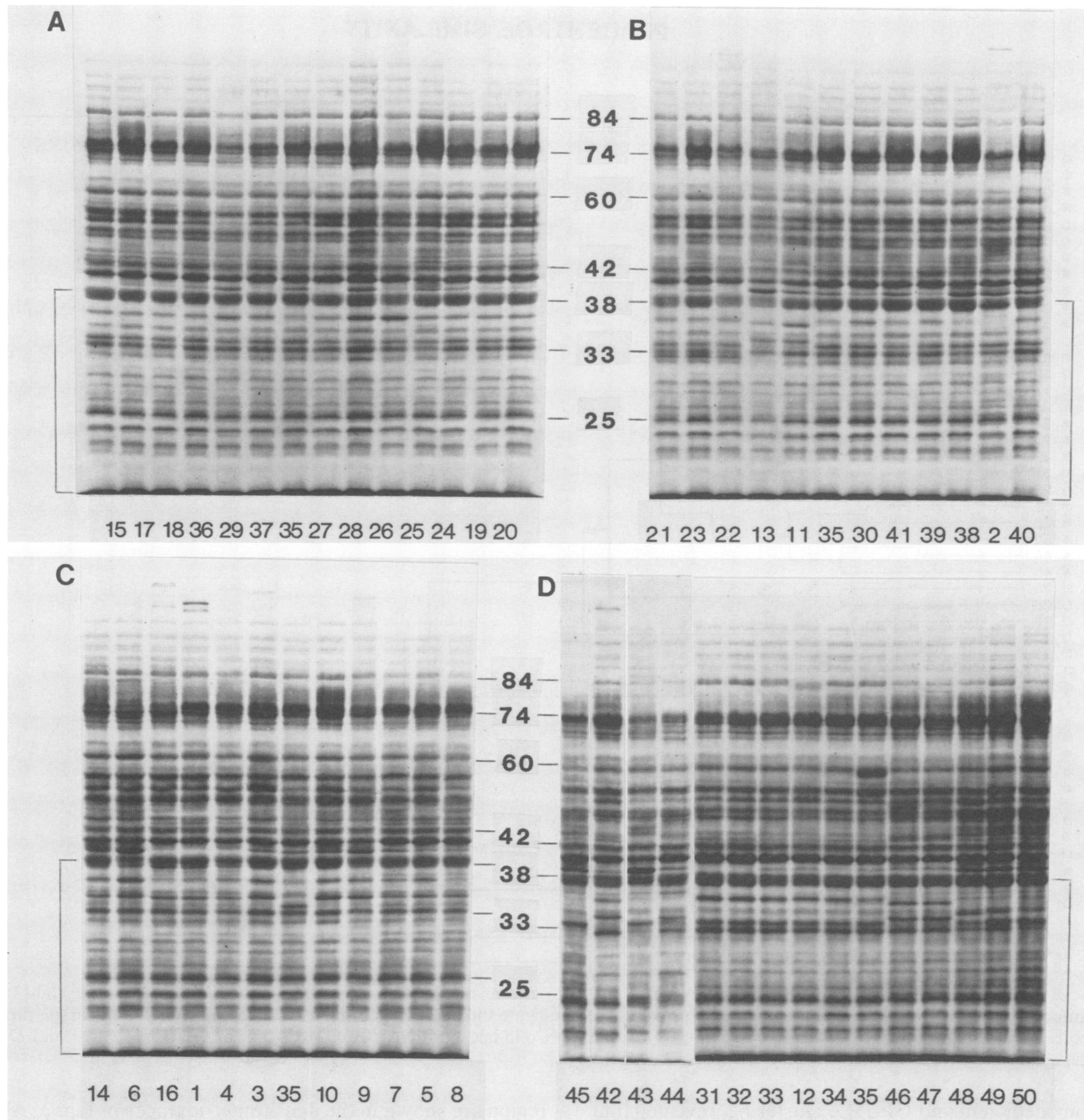

FIG. 1. (A to D) Electrophoretic protein patterns of *S. aureus* (MRSA and MSSA strains). The numbers at the bottom refer to the strain reference numbers used in Table 1 and Fig. 2. Numbers between the gels indicate the molecular sizes (in kilodaltons) of many of the marker proteins used in the calibration of gels. The brackets next to the gels indicate the 18- to 38.5-kDa molecular size range used to define the partial patterns for numerical analysis.

not resolved under the electrophoretic conditions used in this study. PAGE patterns are illustrated in Fig. 1. The total protein profiles were, in general, very similar, with qualitative differences in patterns found principally in the protein bands with molecular sizes in the range of 32 to 38.5 kDa, which is referred to here as the hypervariable region. Fewer interstrain qualitative differences were evident in the 38.5- to 100-kDa region, in which there were multiple bands with larger quantities of focused but less well resolved proteins. The molecular sizes of a number of the marker proteins used in the analysis are given in Fig. 1.

Reproducibility. The protein patterns of the *S. aureus* strains were highly reproducible both within and between gels. Samples of the reference study strain, STH 4696 (isolate 35), which were run on each gel, were used to check the reproducibility and gave an average similarity of 95.6 ± 2.5%. In addition, to check the stability of the protein patterns, replicate samples of eight strains, which reflected the full diversity of the patterns, were made and run on different gels. The average similarity of this set of strains with their respective duplicates was 92.5 ± 2.1%. The phenons that were recognized proved to be extremely robust when the computations were repeated by using different levels of trace alignment and background subtraction.

Numerical analysis. Numerical analysis of partial PAGE protein patterns, based on the determination of the Pearson

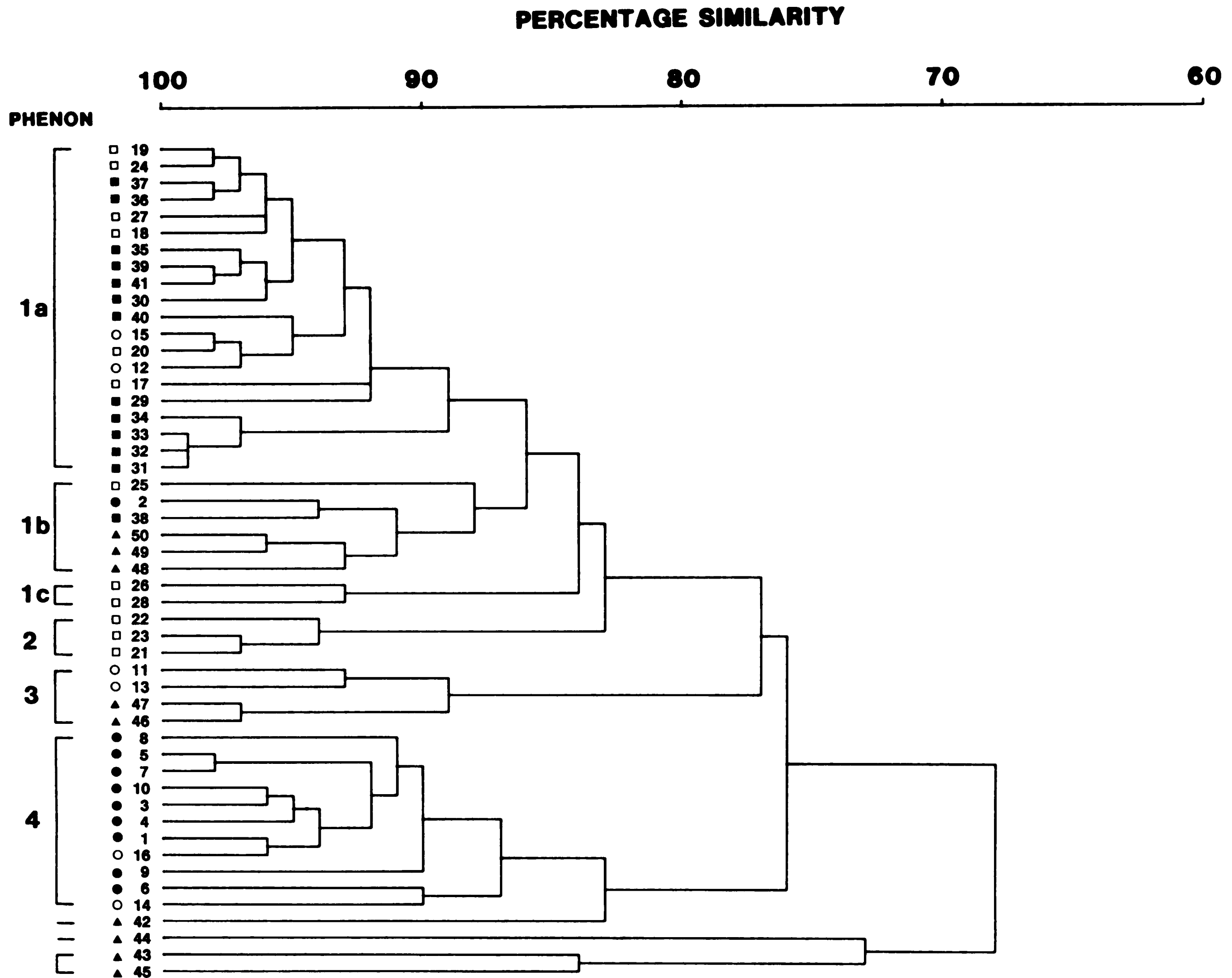

FIG. 2. Dendrogram of the cluster analysis based on partial protein patterns of the strains (isolate numbers are given to the right of the phenon numbers) listed in Table 1. The numbers on the horizontal axis indicate the percentage similarities determined by the Pearson product moment correlation coefficient and UPGMA clustering. Symbols: ●, Colindale OMRSA; ○, OMRSA from United Medical and Dental Schools; □, eastern Australian MRSA; ■, the EMRSA; ▲, MSSA.

correlation coefficient and UPGMA clustering, revealed four clusters of three or more strains of MRSA at the 83% similarity level (Fig. 2).

Phenon 1 contained the majority of the strains tested (28 of 50) but had the lowest intraphenon average similarity (88.7% similarity) and the highest standard deviation (6.1%), which was a reflection of the degree of protein pattern heterogeneity. Comparison at the 86% similarity level revealed a total of six phenons, resulting from the division of phenon 1 into subphenons 1a, 1b, and 1c, mainly because of quantitative differences in what appeared to be common bands (shared bands with similar molecular weights).

Phenons 2, 3, and 4 contained 3, 4, and 11 strains, respectively. Heterogeneity in band pattern, both qualitative and quantitative, was also evident in these phenons, especially phenon 4. The average similarities between the various phenons and subphenons are listed in Table 2. Visual comparisons of protein profiles showed that many of the differences between the profiles were in bands in the hypervariable region. Examples of typical patterns of the six phenons and subphenons illustrating the differences detected in this region are shown in the densitometric traces in Fig. 3. About 10 different prominent bands were discernible, in addition to a number of minor bands, although the assignment of strains to a phenon could be made on the basis of 4 or 5 of the more intense bands. The phenons were coded sequentially from the top of the phenogram in Fig. 2 and were comprised as follows (see Table 1 for strain designations).

(i) Phenon 1. Phenon 1 contained 28 strains: 20 in subphenon 1a, 6 in subphenon 1b, and 2 in subphenon 1c. A total of 12 of the 13 isolates of the EMRSA, with a number of variations in the phage sensitivity pattern, resistogram, and plasmid content, were in subphenon 1a, which also contained two OMRSA isolates from the United Medical and Dental Schools. One of these OMRSA isolates (isolate 15) was isolated from a patient who was transferred from a hospital in Italy. Two of the six MRSA isolates from Australia that were also in this subphenon were indistinguishable by resistogram and phage typing from the classical EMRSA; but the other four of these MRSA isolates had different phage sensitivity patterns, and in addition, two were neomycin resistant. The subphenon designations of

TABLE 2. Mean intra- and interphenon percent similarities determined by the Pearson product moment correlation coefficient (r) and UPGMA clustering[a]

Subphenon or phenon	% Similarity[b]					
	Subphenon 1a (n = 20)	Subphenon 1b (n = 6)	Subphenon 1c (n = 2)	Phenon 2 (n = 3)	Phenon 3 (n = 4)	Phenon 4 (n = 11)
1a	92.3 ± 3.4					
1b	85.6 ± 4.8	90.7 ± 3.4				
1c	84.9 ± 4.7	73.3 ± 7.6	93.0 ± 0.0			
2	84.3 ± 4.5	73.3 ± 6.0	74.3 ± 4.6	94.7 ± 2.6		
3	78.4 ± 3.1	73.1 ± 3.7	75.2 ± 5.2	72.1 ± 5.7	90.8 ± 3.5	
4	77.8 ± 5.1	73.7 ± 7.9	68.3 ± 5.6	72.2 ± 5.6	73.8 ± 3.6	90.1 ± 4.2

[a] Phenons and subphenons were formed at the 86% similarity level.
[b] n is the number of strains in each subphenon or phenon. Values are means ± standard deviations of the similarity estimates.

four of the EMRSA strains that lysogenized with J phage did not change, even though two of the strains had a change in their phage sensitivity patterns (loss of phage 88A in one strain and phages 83A, 84, and 85 in the other). Additional strains of the EMRSA from three outbreaks (5) were all found to give patterns typical of subphenon 1a.

Although the remaining EMRSA strain clustered in a separate subphenon, subphenon 1b, it nevertheless showed a very high similarity to the strains found in subphenon 1a (average of 85.6%). Subphenon 1b also contained one Colindale OMRSA isolate, one eastern Australian MRSA isolate, and three MSSA isolates that were isolated from the same patient and that had the same phage sensitivity patterns.

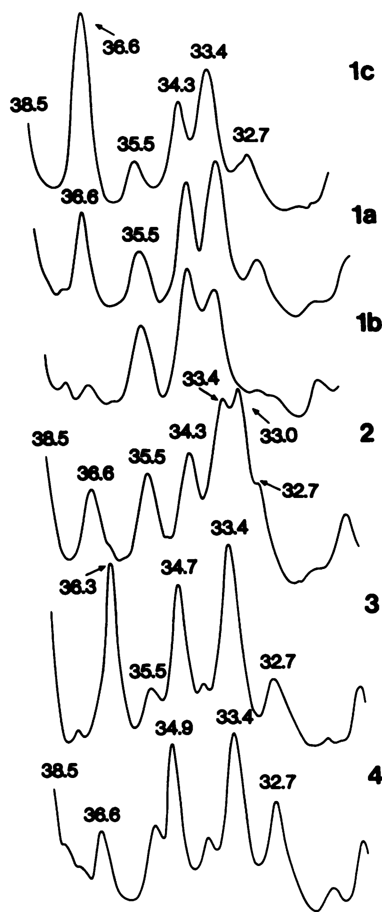

FIG. 3. Densitometric traces of the hypervariable region (32 to 38.5 kDa) of protein profiles representing the six phenons and subphenons of the *S. aureus* strains examined in this study. Molecular sizes (in kilodaltons) of the principal protein bands are indicated.

Subphenon 1c contained two eastern Australian MRSA isolates which differed from the EMRSA isolates in that they were nontypeable by phages; one isolate was resistant to neomycin.

A consistent pattern of protein bands was found in all phenon 1 strains within the 32- to 38.5-kDa region and consisted of bands at approximately 32.7, 33.4, 34.3, 35.5, and 36.6 kDa. The bands at 33.4 and 34.3 kDa were the most intense and gave the phenon 1 pattern its distinct double-band appearance. In addition, both strains that clustered in phenon 1c (isolates 26 and 28) had a noticeably more intense band at 36.6 kDa than those found in other phenon 1 strains. A number of strains included in phenon 1b either had very weak bands (isolates 2 and 38) or did not have bands in this position (isolates 48, 49, and 50).

(ii) Phenon 2. Phenon 2 contained only three strains, all of which were eastern Australian MRSA isolates. The band patterns associated with phenon 2 strains was essentially similar to those of phenon 1, with which they showed a high similarity (average of 81.2%). However, the patterns were characterized by an additional intense band at 33 kDa which gave the phenon 2 strains a triplet of intense bands in the hypervariable region. Two of the three strains were indistinguishable, in phage type and resistotype, from the EMRSA isolates, but the remaining strain (isolate 23) differed in its reactions with the experimental phages.

(iii) Phenon 3. Phenon 3 comprised two strains of OMRSA isolated from different subjects involved in a single outbreak at St. Thomas' Hospital together with two MSSA strains from different subjects. A distinct pattern of bands at 33.4, 34.7, 35.5, and 36.3 kDa characterized the strains that belonged to this phenon. The band at 35.5 kDa was markedly weaker in intensity than the others. All four strains in phenon 3 were sensitive to the group V phages 94, 96, or both. The two MRSA isolates were not conventionally methicillin resistant but were high-level-β-lactamase producers in that they were susceptible to amoxicillin and clavulanic acid (21). We subsequently analyzed another MSSA strain and a similar MRSA strain. Both strains were reactive with group V phages, and each strain gave the protein pattern typical of strains in this phenon.

(iv) Phenon 4. Phenon 4 contained 11 strains, all of which were OMRSA; 9 were from the Colindale set and 2 were from United Medical and Dental Schools. Typically, the most intense bands in the hypervariable region were at 33.4, 34.9, and 36.6 kDa, with additional weaker bands found at

34.3 and 35.5 kDa. Some of the strains (isolates 1, 4, and 16) belonging to this phenon also had a triplet of finer bands specifically in the region from 36.6 to 38.5 kDa. Three other strains (isolates 5, 7, and 8) had a single additional fine band in this same region.

The remaining strains comprised four MSSA isolates, two of which (isolates 43 and 45) gave a pattern characterized by a single major protein band in the hypervariable region and were therefore clustered together at the 83% similarity level. The other two MSSA strains (isolates 42 and 44) gave distinct patterns and remained unclustered. Three additional MSSA strains that had phage patterns that were not included in patterns represented by the original set of strains were also analyzed but produced no new band patterns. Two reacted with phage group II and were in subphenons 1b and 1c, and the third, which reacted solely with phage 95, was in subphenon 1c.

DISCUSSION

Stephenson et al. (26), in their study of MRSA isolates, defined a distinct pattern as one with either one or more [^{35}S]methionine-labeled protein bands with different molecular weights or one with two or more bands with the same molecular weight but with pronounced differences in intensity. Other investigators (11, 17) have also interpreted their profiles visually but have not specified any conditions for the definition of the difference between patterns and, as with Stephenson et al. (26), were unable to compare profiles between different gels. More recently, the Dice coefficient has been used to estimate the similarity between profiles after a visual interpretation of the patterns (27); no definition of the level of staining required to identify a band was given, and again, there was no clear discrimination between isolates. In the present study, we avoided a visual interpretation of patterns, which may be subjective, but used a high-resolution laser densitometer to record both the quantitative and qualitative data needed to define a pattern. We then expressed the similarity between patterns using a correlation coefficient. The high resolution of our SDS-polyacrylamide gels, coupled with the facility for between-gel correction in the computer program, enabled us to make an objective comparison of the profiles produced on different gels, something which has not previously been achieved (11, 17, 26).

The results of our intergel analysis of protein patterns by conventionally stained SDS-PAGE were very similar to those of Stephenson et al. (26) for an identical set of strains, despite the difficulties outlined above. In the present study all of the EMRSA isolates clustered together in phenon 1; all but one isolate fell within the same subphenon, subphenon 1a. Similarly, with the exception of a single strain (isolate 2) the Colindale OMRSA isolate clustered in a single phenon, phenon 4.

We also examined five strains from the study of Krikler et al. (17). They were unable to distinguish between these strains by SDS-PAGE of whole-cell extracts, but our results obtained by this technique were in agreement with their analysis of immunoblots of culture supernatants. Two of these strains (isolates 46 and 47) were from different subjects, but both clustered within phenon 3. Phenon 3 may be exclusive to phage group V strains, in that it was made up solely of strains of this phage group (four strains from the initial study and two additional strains). It is interesting that Krikler et al. (17) also found that the two strains were indistinguishable from each other but were different from strains from their other subjects. Phage group V strains are also known to be distinct in their restriction modification system and other properties (1).

There appeared to be no correlation between protein type and resistotype or phage pattern. The resistotype is known to vary with the loss or gain of transposons or plasmids (19), and the phage sensitivity pattern can similarly be altered by lysogenization with phages (2). The plasmid content did not appear to correlate with the protein pattern of our EMRSA isolates. The protein pattern was not altered even after strains were lysogenized with J phage.

The SDS-PAGE pattern may be an additional property that could be used in a hierarchical typing system. Isolates of the EMRSA appeared to be closely related, although they did not exclusively make up subphenon 1a, since subphenon 1a also included two OMRSA isolates from United Medical and Dental Schools. Some eastern Australian MRSA isolates that had identical phage patterns and resistotypes were also in subphenon 1a (e.g., isolate 20), but others that also had epidemic potential (M. Beard-Pegler, personal communication) were not. We speculate that the SDS-PAGE protein pattern is a marker of a more stable underlying clonal pattern. The EMRSA isolates from southeast England may thus have originated from a strain that was indistinguishable from some of the eastern Australian MRSA isolates. The plasmid for gentamicin and resistance to nucleic acid-binding compounds may have spread to other strains in the United Kingdom, and a greater variety of protein patterns may be seen, as in the MRSA isolates from eastern Australia.

Lacey and Grinstead (18) have previously proposed a clonal hypothesis for the evolution of MRSA isolates, suggesting that all MRSA isolates are derived from a single ancestor. Interestingly, NCTC 10442, one of the first described MRSA strains (15), gave a different protein profile (phenon 4) from that of the more recently described eastern Australian MRSA and the EMRSA isolates (phenons 1 and 2), which others have suggested may be related (5, 28, 29). Therefore, they appear to have evolved sufficiently along divergent paths to be considered distinct clones based on their protein patterns.

In previous studies in which typing has been investigated by SDS-PAGE of proteins (7, 22) within a single species, a larger number of phenons has been evident. Despite the highly defined nature of the majority of the test strains under investigation in this study (i.e., MRSA strains), by the methods described, we were able to group the strains objectively and reproducibly into four phenons. The underlying pattern of bands may represent "fingerprints," and it may be possible to define additional groups and thus increase the discriminatory power of the method. The portability of the method was demonstrated by a similar analysis on separately prepared samples at St. Thomas' Hospital. In that case, it was possible to assign strains to the four phenons by a visual inspection of the gels by using criteria that were established in the computer-derived analysis. In any case, all strains were typeable by this method, and it has additional advantages of stability over both phage typing and resistotyping.

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